



Supplementary Figure 1

Supplementary Information Legend

Sup figure 1. Appearance of hyaluronan-bound QDs precedes integrin-mediated adhesion by minutes. A stable cell line of chondrocytes, RCJ-P, expressing paxillin-GFP fusion protein was labeled with biotinylated hyaluronan binding proteins and streptavidin conjugated quantum dots (QDs) and seeded on a glass-bottom culture dishes, pre-coated with 10 μ g/ml fibronectin (A and B) or with fetal calf serum (C and D). Time-lapse movies were immediately recorded using two-colour TIRF microscopy, acquiring an image every 15 sec for 30 min. (A) On fibronectin-coated surface QDs appeared 1 min after seeding, marked here as time zero (red, arrowheads). (B) Paxillin-GFP became visible only 1.5 min later (green, arrow). (C) On serum-coated surface QDs appeared 4 min after seeding, marked here as time zero (red, arrowheads). (D) Paxillin-GFP became visible only 2 min later (green, arrow). Note that on both surfaces QDs and Paxillin-GFP structures are spatially segregated. Scale bar = 2 μ m.

Sup movie 1. Appearance of hyaluronan-bound QDs precedes integrin-mediated adhesion by at least 2-10 min. A stable line of chondrocytes, RCJ-P, expressing paxillin-GFP fusion protein was labelled with biotinylated hyaluronan binding protein and streptavidin-conjugated quantum dots (QDs). Labelled cells were plated on a glass bottom culture dish and time-lapse movies were immediately taken using two-colour TIRF microscopy, acquiring an image every 15 sec for 75 min. GFP is green and QDs are red, scale bar= 2 μ m. (MPEG, 2.7 MB)

Sup movie 2. Vertical-movement of Quantum dots. QDs intensity during cell adhesion is depicted as 3D graphs. Images were acquired every 15 seconds and the exposure time was 500 ms. The cell periphery is outlined in red. More QDs are visible as the cells spread, but they are less dynamic. (MPEG, 1.8 MB)

Sup movie 3. Reorganization of QDs under spreading cells. QDs are represented by coloured pixels, all pixels are plotted in cumulative manner such that all the coordinates in which QDs appeared within the given time span are marked with a coloured pixel. Paxillin containing structures are white, pixel colours indicate the time frame of

appearance for each QD: 0-20 min (red), 21-40 min (blue) 41-55 min (green), new QDs in every frame are yellow. (MPEG, 1.7 MB)

Sup movie 4. Hyaluronan modulates initiation of paxillin-containing structures. A line of RCJ-P chondrocytes, stably expressing paxillin-GFP, were treated with hyaluronidase (right) or left untreated (left). The cells were then plated on a glass bottom culture dish and time-lapse movies were immediately taken using TIRF microscopy. Images were acquired every 15 sec for 15 min, Scale bar= 2 μ m. (MPEG, 2 MB)